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RADIATION DAMAGE IN MUSCLE CELL MEMBRANES AND REGULATION OF CELL METABOLISM

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ABSTRACT

The absence of detectable early effects on frog striated muscle of low doses (under 10,000r) of X-rays has already been reported. Effects of higher (100,000r) doses include prolonged relaxation time, more rapid fatigue, and decreased ATP and glycogen content, but increased potassium and sodium effluxes, sodium influx and oxygen consumption, compared with non-irradiated controls. Twitch latency and rise time are not changed. Magnesium-activated ATPase activity of homogenates or mitochondrial suspensions decreases immediately afted irradiation, but increases with time more rapidly than in controls. The biochemical findings suggest uncoupling of oxidative phosphorylation, while the ion flux data suggest increased membrane permeability. We, therefore, adopt the working hypothesis that irradiation results in membrane damage, both in the sarcoplasma membrane, thereby increasing ion permeabilities, and in the mitochondrial structure, thereby interfering with oxidative phosphorylation and reducing ATP production. A decrease in membrane potential during irradiation to about 90% of its control value has already been reported. The observed increases in Na and K fluxes after irradiation explain these findings. At these radiation levels, our data do not require any hypothesis of early direct damage to the contractile mechanism per se.

INTRODUCTION

We have reported (1) that low doses of X-rays (50 to 10,000r) have no significant early effects on frog skeletal muscle, whereas higher doses (50,000 to 100,000r) increase the potassium efflux and reduce the membrane potential. Gerstner, et. al (2) have also reported some early effects of 100,000r doses on frog gastrocnemius muscle. Their findings included decreased contraction amplitude for heavy loads, prolongation of relaxation time, and more rapid onset of fatigue in irradiated muscles. Latency and rise time of contraction were not affected. Since all their results suggested a lowered ATP level, these workers concluded that radiation primarily attacks oxidative metabolism rather than the contractile mechanism per se.

The present study is a continuation of our earlier work at 100,000r. The results corroborate and supplement the findings of Gerstner, et al. and support the hypothesis that one of the early effects produced by high doses of X-ray is damage to membranes. Damage to the plasma membrane affects ion transport systems, while damage to mitochondrial membranes interferes with the conversion of oxidative energy to phosphate bond energy. High doses of X-rays, therefore, produce partial uncoupling of oxidation and phosphorylation, increasing respiration and allowing the accumulation of ADP. The rate of glycolysis is thereby accelerated, but since glycolysis alone cannot provide sufficient ATP to satisfy the energy needs of the cell, the ATP level drops. No evidence requiring the hypothesis of direct early damage to the contractile system was obtained.

METHODS

These data were obtained during the period from September 1959 to May 1962. For all studies, sartorius or gastrocnemius muscles of Rana pipiens were dissected carefully, weighed and identified in left-right pairs. One member of each pair was used as a control, the other was irradiated. Only sartorius muscles with fresh weights in the rage from 38 to 45 mg and gastrocnemius muscles with weights from 850 to 900 mg were used. After dissection and weighing, muscles were soaked at 25°C for either 2 or 5 hours, as required, in potassium-free Ringer's solution of the following composition: NaCl, 112mM; $CaCl_2$, 1.9mM; Na_2HPO_4, 2.5mM; NaH_2PO_4, 0.5mM; pH 7.2-7.4.

Muscles were irradiated at 25°C in plastic chambers at 6,000r/min,m to a total dose of 100,000r with a G. E. Maxitron X-Ray machine, running at 300 KVP to 20 ma, with 0.25 mm Al filter. Dosage was calibrated with a Victoreen Radocon dosimeter. Control muscles were treated identically except that they occupied similar plastic chambers outside the irradiation room.

For biochemical studies, muscles were pooled in groups of six or nine, and homogenized in 9 volumes of cold 0.25M sucrose solution.

1. Potassium loss

Experimental groups of muscles were irradiated as above after soaking for 2 hours. Control as well as experimental groups were then soaked in 10 ml of fresh K-free Ringer's for appropriate periods of time at 0°C or 25°C. After removal of the muscles the potassium content of each soaking solution was determined by flame photometry. (3) 720 control and 720 irradiated muscles were studied in groups of 6.

2. **Sodium fluxes**

a) Sodium influx: 46 control and irradiated pairs of sartorius muscles were studied. Each muscle after soaking in K-free Ringer's for 2 hours, was mounted at its rest length in a special holder and placed for 20 minutes in K-free Ringer's with a Na^{22} content of 6.2×10^5 CPM/ml. Each of these muscles was then removed from the solution and its Na^{22} content determined with a Gamma spectrometer with scintillation well counter. Back-ground was 1CPM. Counting took less than 1 minute. Immediately on replacing in the Na^{22} solution, experimental muscles were irradiated. Na^{22} content of control and experimental muscles was then determined at intervals up to 7 hours after irradiation.

b) Sodium efflux: 46 pairs of control and irradiated muscles were studied. Sartorius muscles, mounted as above, were loaded with Na^{22} by soaking for 5 hours at 25^oC in K-free Ringer's containing Na^{22}. Experimental muscles were irradiated at the end of this period. The Na^{22} content of each muscle was determined immediately after irradiation. Each muscle was then placed in 5.5 ml of non-radioactive K-free Ringer's solution, and the Na^{22} content of these bathing solutions determined as a function of time up to 3 hours after irradiation. The calculation of ionic fluxes were done according to Keynes' methods (4) and those of Portela, et al. (1).

3. Mechanical Parameters

Length-force diagrams in the contracted state, latency and shape of single maximal twitches, and time to develop contracture in response to prolonged stimulation were studied following the procedures of Gerstner, et al. (2) and Portela, et al. (1). For

single twitches, 30 control and 30 irradiated sartorius muscles were stimulated to lift a load of 32 gm by rectangular pulses of 1.5 ma and 1 msec. duration, repeated at 6 second intervals. Length-force diagrams in the contracted state were studied in 32 pairs of gastrocnemius muscles, stimulated with 30 cps sine wave stimuli, 1 volt amplitude, in 1 second bursts. Loads of 32, 75, 150 and 300 gm were used to stretch the muscles.

4. Electron Microscopy

After irradiation, pools of muscles were soaked in K-free Ringer's at 25°C for periods up to 7 hours. At appropriate intervals, control and irradiated pairs were fixed for 2 - 3 hours in cold 1% osmic acid buffered to pH 7.4 with 0.02M Na-Veronal-HCl buffer, and embedded in methacrylate following conventional procedures (5). Sections were cut on a Porter-Blum ultramicrotome and examined in an RCA EMU-3 electron microscope.

5. ATP-ase activity

Pools of 6 muscles were homogenized in 9 volumes of cold 0.25M sucrose solution, and the homogenate used at once for ATP-ase assay by the method of Dubois and Potter (6). Assays were run at 37°C for 10 minutes with amounts of homogenate equivalent to 8.2, 17 and 25 mg of muscle, in the presence and absence of 10^{-3}M $CaCl_2$ and $MgCl_2$ in a final volume of 1.9 ml. Phosphate was determined by the method of Fiske and Subbarow (7). Assays were run immediately after irradiation, and on muscles soaked in 25°C K-free Ringer's for 5 hours after irradiation. In some experiments, mitochondrial suspensions were prepared from homogenates of 30 muscles each following the procedure of Chappell and Perry (8) and their ATP-ase activities determined similarly.

6. Oxygen consumption

Oxygen consumption of whole sartorius muscles was measured at 27°C in a conventional Warburg apparatus, following the general procedure of Keynes (9). K-free Ringer's or 2.5 mM KCl-Ringer's was used. 256 muscles were analyzed.

7. Oxidative phosphorylation

P/O ratios were determined on mitochondrial suspensions at 27°C following the procedure of Hunter (10). The 3 ml of reaction mixture in each flask contained 0.013M K_2HPO_4; 1.2×10^{-5}M cytochrome C; 6×10^{-3}M $MgCl_2$; 1.7×10^{-3}M ATP; 0.02M Glucose; 0.01M NaF; 3.5×10^{-4}M DPN; 0.01M Na-succinate; 422ugm hexokinase (Sigma type IV); and 1 ml of mitochondrial suspension prepared from an homogenate of 30 muscles. All were kept at pH 7.4. 0.4 ml of 40% w/v trichloracetic acid was added to the reaction mixture from a side arm after 8 minutes equilibration for the zero-time flasks and after 38 minutes for the 30 minute flasks. All samples were centrifuged and the supernates analyzed for phosphate following the procedure of Lowry and Lopez (11).

8. Succinic dehydrogenase activity

Succinic dehydrogenase activity in homogenates was determined in a Warburn apparatus at 27°C following the technique of Schneider (12). 3 ml of reaction mixture in each flask contained: 1.3×10^{-5}M cytochrome C; 0.05M Na- succinate; 0.033 M Na_2HPO_4; 4×10^{-4}M $CaCl_2$; 4×10^{-4}M $AlCl_3$; 1.2×10^{-3}M AMP; 1.2×10^{-3}M ATP volumes of homogenate equivalent to 33, 48 and 84 mg of muscle at pH 7.4. 96 pairs of muscles were studied in groups of 6.

9. Proteolytic activity

Following the procedure of Anson (13) as modified by Snoke and Newraht (14), the amount of breakdown added hemoglobin at pH 4.0 was used as a measure of the proteolytic activity of homogenates. 22 control and 22 irradiated pools of 9 muscles each were studied. 1 ml of a 2% solution of hemoglobin in a 0.2 M Na-acetate at pH 4.0 and 0.8 ml of a 10% muscle homogenate in 0.25M sucrose brought to pH 4.0 with 0.25N HCl were mixed and brought to a total volume of 2.0 ml with 0.2M Na-acetate. After incubation at 37°C for 2, 4 or 6 hours, 2 ml of 10% w/v trichloracetic acid were added to stop the reaction, the reaction mixture centrifuged, and the optical density of the supernate determined at 280 mu on a Beckman DU spectrophotometer.

10. ATP assay

ATP content of individual gastrocnemius muscles was determined by the firefly luminescence technique of Strehler and Totter (15). This method is based on the linear luminescence response of firefly extracts to added ATP. Each muscle was homogenized in cold 0.4M perchloric acid and centrifuged at 1,000xg for 10 minutes. The supernate was neutralized with KOH and diluted as needed with 0.06M perchloric acid to a final equivalent concentration of tissue of 1 mg per ml. Control runs showed that perchloric acid extraction did not alter the ATP content of the homogenates. Samples were run with internal ATP standards. Luminescence was measured in a special photometer. An accuracy of 2% and sensitivity of 0.1 ugm of ATP were achieved.

128 muscles were studied under a variety of conditions. Loads

of 32, 75, 180 and 380 gms were used. For each load, muscles were treated in the following ways: 1. Unstimulated, control; 2. unstimulated but irradiated before loading; 3. stimulated once every 20 seconds with maximal stimulus (1.5 ma, 1 msec rectangular pulse) but not irradiated; 4. stimulated as in 3. and irradiated before loading. During the 1 hour loading period, muscles were kept moist in a plastic chamber partially filled with Ringer's through which oxygen was bubbled.

11. Glycogen content

Glycogen was determined in muscles subjected to a load of 32 gm, under experimental conditions similar to those described for ATP determinations. 30 control and 30 irradiated muscles were analyzed. Glycogen was extracted from individual gastrocnemius muscles by digestion in 30 per cent KOH for 1 hour in a boiling water bath. Glycogen was precipitated from the digest with 95% alcohol, centrifuged, hydrolyzed in 0.6 N HCl and neutralyzed with NaOH, using phenol red as indicator. Glucose was determined in the hydrolysate by the method of Somogyi (16).

RESULTS:

1. Potassium loss

The results obtained summarized in figure 1 and table 1, agree with those previously published (1). The finding that the relative increase in the rate of potassium loss produced by irradiation is the same at 0°C as at 25°C is interesting because it suggests that the effect is not on metabolic reactions but on membrane structure. In any case, 100,000r increases the rate of potassium loss from frog sartorius muscles in K-free Ringer's solution by approximately 50%.

The experiments at 25°C were carried out during 1959-62, and those at 0°C during 1961-62. It will, therefore, be necessary to test the validity of the 2.5 ratio between the potassium effluxes at these two temperatures by carrying out simultaneous experiments. Since the data presented in table 1 result from analyses of 720 muscles, at all seasons of the year, however, significant changes are not expected.

2. Sodium fluxes

As indicated in figure 2, Sodium uptake increased during the irradiation period. The figures for sodium efflux in table 2 show a significant increase following irradiation.

3. Mechanical parameters

The general results of these experiments agree with those of Gerstner, et al. (2). Maximal contraction amplitude under load, as indicated by analysis of the length-force diagrams, decreases significantly in irradiated muscles. The latent periods and rise times of single twitches are not significantly different in control and irradiated muscles. In control muscles relaxation time, defined as the time from the peak of the twitch to 1/10th peak displacement, averaged 42msec. and did not change during 20 minutes of stimulation. In irradiated muscles, the relaxation time increased and the contraction height decreased after only 10 minutes of stimulation. Contracture is seen in control muscles only after 30 to 50 minutes of stimulation, and is generally reversible. In irradiated muscles, contracture is seen after 20 to 30 minutes of stimulation and is generally irreversible. The results are summarized in tables 3 and 4.

4. Electron microscopy

Soaking for several hours in Ringer's solution at 25°C leads to swelling and progressive deterioration of myofilaments, sarcoplasmic

reticulum and mitochondrial structure, in both control and irradiated muscles. However, the irradiated muscles consistently show much more rapid and more extensive alteration of the structure of mitochondrial cristae and membranes than do controls (figures 3 and 4). A more detailed analysis of these particular results will be presented elsewhere.

5. ATP-ase activity

The data are summarized in table 5. (Each figure in this table is an average of 10 determinations). ATP-ase activity of muscle homogenates without added calcium or magnesium is not significantly changed by irradiation. Addition of 10^{-3}M Ca^{++} approximately doubles the ATP-ase activity in homogenates of both control and irradiated muscles. Addition of 10^{-3}M Mg^{++} approximately triples the ATP-ase activity in control homogenates, and this effect is depressed significantly immediately after irradiation. Homogenates prepared from irradiated muscles 5 hours after irradiation show the same level of Mg^{++} activated ATP-ase as controls, however (see figure 5). The Mg^{++} activated ATP-ase of mitochondrial preparations is the same in control and irradiated material immediately after irradiation, but after 5 hours is significantly higher in irradiated than in control preparations.

These results should be compared with the "ageing" effects observed in studies of mitochondrial preparations. When conventional mitochondrial suspensions are allowed to stand for several hours, their oxidative phosphorylation and DNP-activated ATP-ase activity decrease. Simultaneously, a Mg^{++} dependent ATP-ase activity appears. The effects of irradiation on muscle ATP-ase activity, therefore, suggest that damage to the mitochondria similar to the deterioration involved in "ageing" may be an important radiation effect. The nature

of the decrease in Mg^{++} activated ATP-ase activity of whole homogenates immediately after irradiation is not yet understood.

6. Oxygen consumption

In agreement with the results of Keynes (9), the oxygen uptake of control resting muscles at 27°C is 86ul O_2/gm muscle/hr in normal potassium Ringer's. Irradiation doubles the rate of oxygen uptake. The results are summarized in figure 6 (each point in the curve is an average of 16 muscles) and table 6. In K-free Ringer's, the oxygen consumption is 40% lower in both control and irradiated muscles, and the effect of irradiation again is to double oxygen uptake.

7. Oxidative phosphorylation

The P/O ratios obtained from mitochondrial suspensions 4 hours after irradiation are 30% lower than those obtained from controls (table 7). (Each value in the table represents one pool of 30 muscles). The failure to obtain normal P/O ratios in irradiated muscle preparations should be compared with the increased Mg activated ATP-ase found in these preparations. Both these sets of data indicate damage at the level of the mitochondrial cristae, and a progressive uncoupling of oxidative phosphorylation there.

8. Succinic dehydrogenase

Table 7 summarizes the results. No significant effect of radiation on the activity of this enzyme system was found.

9. Proteolytic activity

As figure 7 shows, proteolytic activity of control homogenates increases steadily with time, but irradiation is without significant effect. The changes in other parameters resulting from irradiation are, therefore, not to be explained merely as a result of autolysis.

10. ATP content

The ATP content of control resting muscles was found to be 2 mg per gm muscle. Irradiation decreases this value by approximately 20%. Stimulation and irradiation decrease it by approximately 30%, whereas stimulation of control muscles under our experimental conditions produced no significant change in ATP content. The results are summarized in table 9.

11. Glycogen content

As table 10 indicates (each value in the table is an average of 6 muscles), the glycogen content is extremely variable. In every case, however, the glycogen content of irradiated muscles is less than that of the corresponding controls. Furthermore, stimulation of irradiated muscles results in twice the glycogen loss observed in stimulated controls.

DISCUSSION

The respiration of muscle cells is a complex process, and its rate can be affected by many factors. Control mechanisms occur at several levels. In particular, the rate of oxygen consumption depends upon the rate at which electrons are transferred from the reduced catalysts of the Electron Transfer System to molecular oxygen. This process is normally coupled directly to phosphorylation of ADP, the level of which in turn controls the rate of glycolysis. If partial uncoupling of phosphorylation from oxidation occurs, the rate of oxygen consumption and the concentration of ADP will both increase, and so will the rate of glycolysis. The rate at which ATP can be supplied will decrease, however. On this basis, the biochemical findings in this study may be fitted into a consistent picture

of early radiation damage in the muscle cell.

In the first place, the electron micrographs indicate much more severe breakdown of the mitochondrial cristae in irradiated cells than in controls. Furthermore, the effects of radiation on ATP content, ATP-ase activity, P/O ratios, and oxygen consumption all suggest partial uncoupling of oxidation from phosphorylation, the enzyme systems for which are known to be intimately associated with the structure of the cristae. The changes in mechanical parameters suggest reduced availability of ATP. The absence of early radiation effects on succinic dehydrogenase activity indicates that the metabolic enzymes of the tricarboxylic acid cycle, which are not directly involved in the cristae, are not damaged.

We propose, therefore, that one of the primary internal "early" effects of 100,000r of X-rays on muscle cells is damage to membrane organization at the level of the mitochondrial cristae. As a result, less ATP is available, phosphorylation and oxidation are partially uncoupled, oxygen consumption increases, ADP content increases, glycolysis increases and glycogen is consumed.

Stimulation of contraction under these conditions will lower the glycogen and ATP content even further, leading to decreased work capacity and irreversible contracture, as observed.

At the level of the plasma membrane (sarcolemma) radiation damage results in increase of potassium and sodium fluxes. In earlier work (1) an increase in membrane conductance and a decrease in membrane potential during and after irradiation were demonstrated. From the known relationships between membrane potential and ion permeability it is clear that this depolarization can be accounted for by the increased sodium permeability demonstrated here.

The increase in sodium efflux (sodium pumping) after irradiation can be compared with the increase in oxygen consumption, as has been done in table 11, but it is not possible to establish any causal connection between them in the light of present knowledge. A wide variety of processes may be stimulated by radiation, and thereby increase the oxygen consumption of the whole cell. At present it is not known what fraction of the increased oxygen consumption is used for increased sodium pumping. Changing the external potassium concentration from 0 to 2.5 mM increases the rate of oxygen consumption 1.6 times in control and 1.7 times in irradiated muscles (table 6). However, the ratio of oxygen consumption in irradiated muscles to oxygen consumption in controls is 2.0 for external potassium concentrations of 0, 2.5, or 10.0 mM (16). These results indicate that irradiation stimulates oxygen consumption independently of external potassium concentration, even though the latter normally exerts a significant influence on the rate of oxygen consumption. Experiments are in progress in this laboratory to determine the effects of higher levels of X-irradiation on the sodium efflux and oxygen consumption in the presence of 0, 2.5, and 10mM potassium, in order to elucidate the interactions between sodium and potassium fluxes and oxygen consumption.

We are also currently analyzing the actions of appropriate metabolic inhibitors (cyanide, DNP, IAA, Ouabain and physostigmine) on the increase of sodium loss from the muscle fibers produced by 100kr, in an attempt to identify the pathways by which metabolic energy drives the sodium pumping mechanisms.

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TABLE I

Ratios between rates of K^+ loss of controls and irradiated muscles obtained at 0°C and 25°C.

C = controls
I = irradiated

K^+ loss in mg K^+/gm hr				1 / C		$C_{25°c} / C_{0°c}$
C		I				
0°C	25°C	0°C	25°C	0°C	25°C	
0.0424	0.1100	0.0660	0.1660	1.57	1.50	2.6

FIGURE I

Rate of K^+ loss (in K^+-free Ringer solution) as a function of time.

--- Experiments at 0°C; o = controls; Δ = irradiated
___ Experiments at 25°C; ● = controls; x = irradiated
I S.E.M.

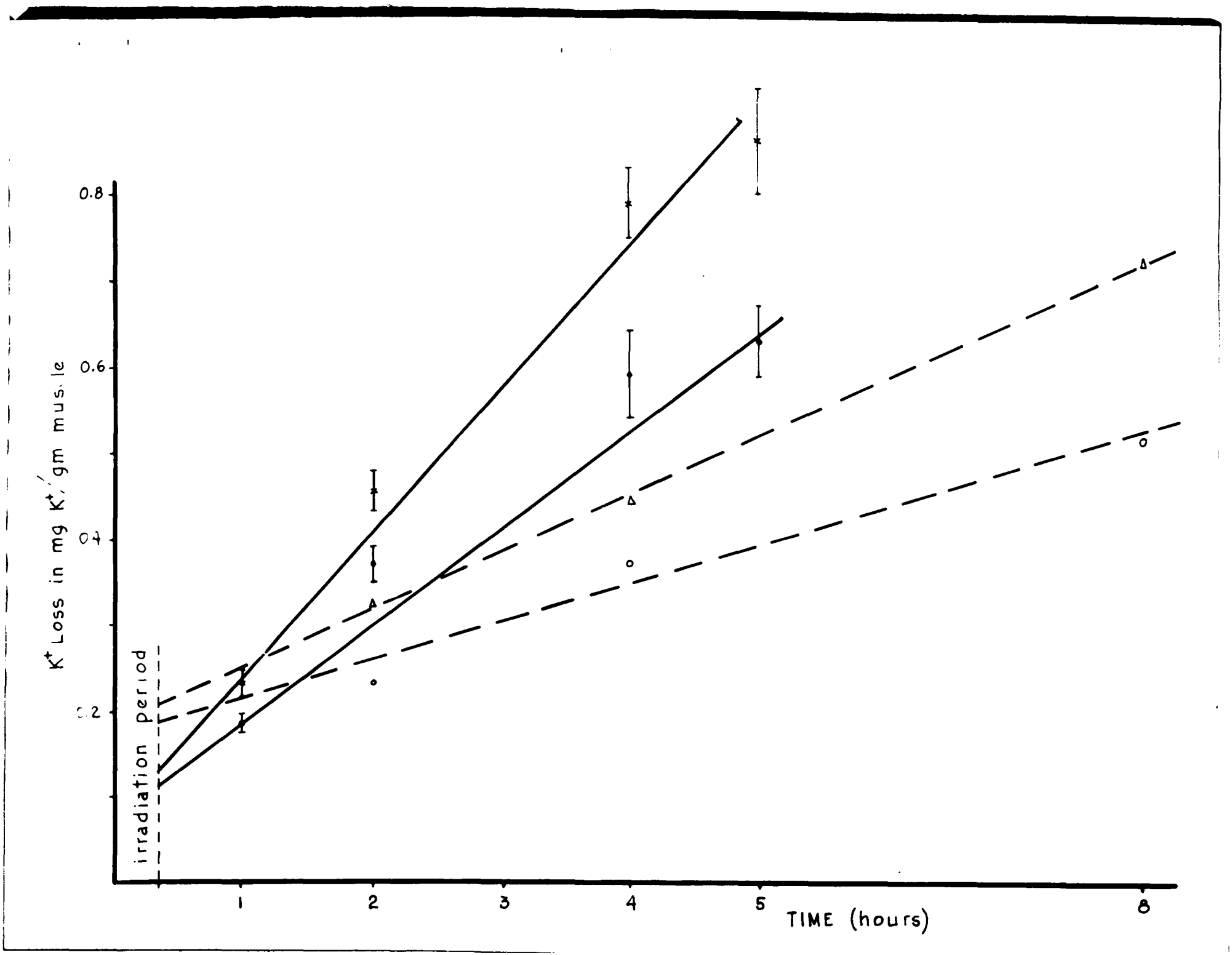
0.8
0.6
0.4
0.2
K+ Loss in mg K+/gm muscle
irradiation period
1
2
3
4
5
6
TIME (hours)

TABLE 2

Na efflus of resting muscles
(in p-moles $cm^{-2}.sec^{-1}$)

SODIUM EFFLUX OF RESTING MUSCLES (in p-Moles.cm^{-2}.sec^{-1})				
No. of muscles	Dose in kr	OUTFLUX		
		CONTROL	IRRAD.	IRRAD./CONTR.
16	100	7.15	9.80	1.37
16	100	7.65	10.10	1.32
8	100	6.22	7.80	1.25
6	100	7.00	8.70	1.24

FIGURE 2

Na^{22} uptake as a function of time

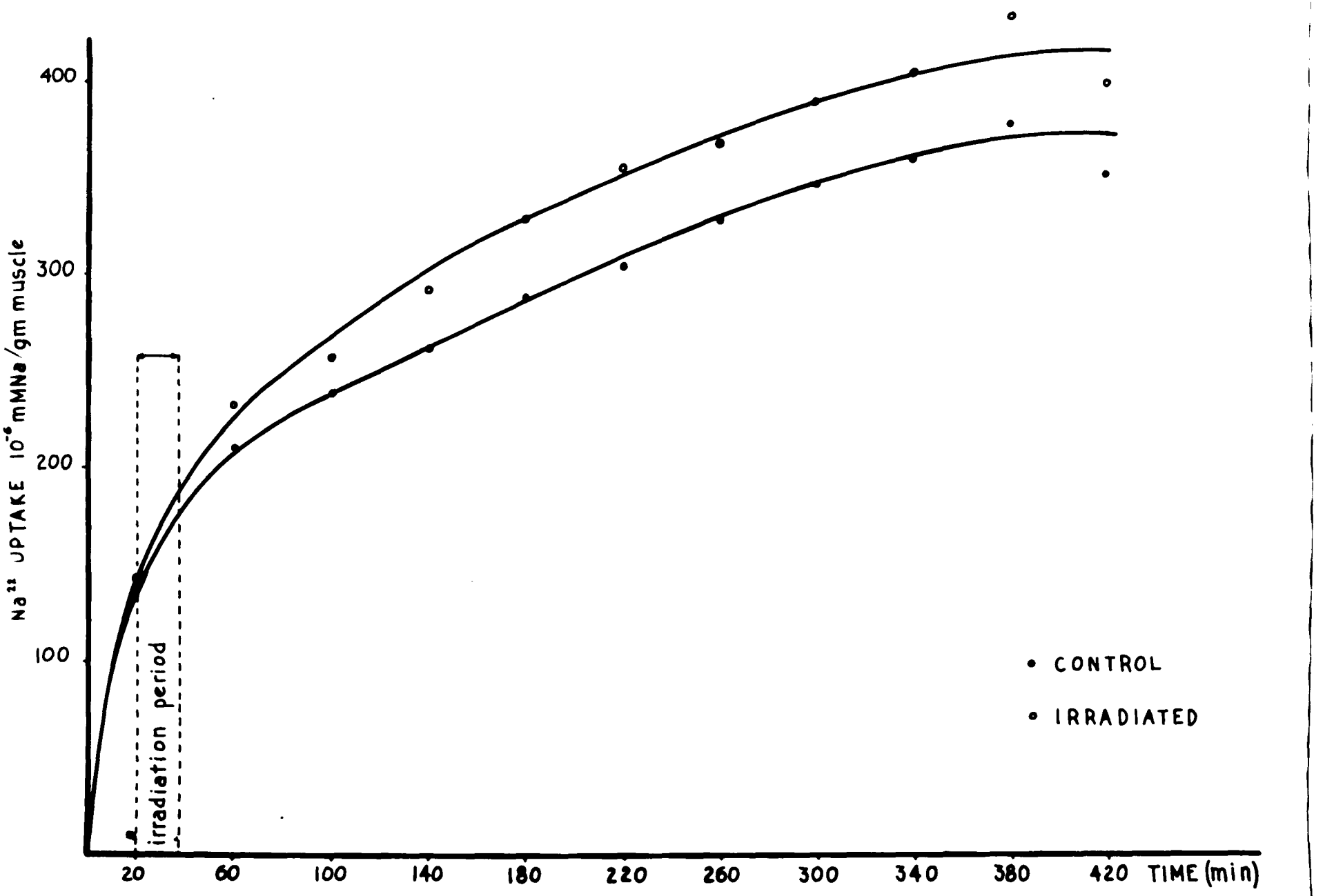
400
300
200
100
Na^{22} UPTAKE 10^{-6} mMNa/gm muscle
irradiation period
20
60
100
140
180
220
260
300
340
380
420
TIME (min)
• CONTROL
• IRRADIATED

TABLE 3

Mechanical response of frog muscles

Fatigue: per cent of muscles that were exhausted and unable to produce twitches

C = controls
I = irradiated

Time in min. after start the stimulation	LATENT PERIOD (msec)		CONTRACTION TIME (msec)		RELAXATION TIME (msec)		FATIGUE	
	C	I	C	I	C	I	C	I
5	9.4	9.0	39	45	40	41	0	0
15	9.1	9.2	43	41	39	54	0	5.7
20	9.3	9.4	40	44	42	78	0	14.3
25	9.2	9.1	40	46	40	90	0	28.6
30	9.0	9.3	45	43	38	>190	0	51.4
40	9.4	∞	43	∞	44	∞	5.7	100.0

TABLE 4

Radiation effects on the length-force diagrams of 32 pairs of Frog muscles.

C = controls
I = irradiated

LOAD IN GRAMS								
CONTRACTION (mm)	30 gm		100 gm		150 gm		300 gm	
	C	I	C	I	C	I	C	I
	7.1	6.8	7.9	7.0	7.4	4.5	6.1	2.9
±SEM	0.8	0.6	0.5	1.0	0.5	2.3	1.4	2.2

TABLE 5

ATPase activity of Frog muscles

C = controls
I = irradiated

ATP-ase ACTIVITY

mg equivalent tissue	WITHOUT ACTIVATOR						10^{-3}M Ca^{++} AS ACTIVATOR						10^{-3}M Mg^{++} AS ACTIVATOR					
	8.5		17		25.5		8.5		17		25.5		8.5		17		25.5	
Post-irrad. soak. period	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I
0 hr	7.0	5.6	15.3	14.4	25.8	22.7	13.4	11.4	20.3	19.3	25.3	22.9	18.7	16.0	25.8	24.6	29.2	29.2
5 hr	5.2	5.0	15.2	13.3	23.3	20.7	13.1	11.9	20.0	19.3	25.2	25.1	19.0	18 5	26.0	25.0	31.3	32.0

FIGURE 5

ATPase activity of whole muscle homogenate and mitochondrial suspension

in muscle homogenate:

A, B and C: controls _____
A', B' and C': irradiated -----

in mitochondrial suspension:

M: controls_____
M': irradiated ------

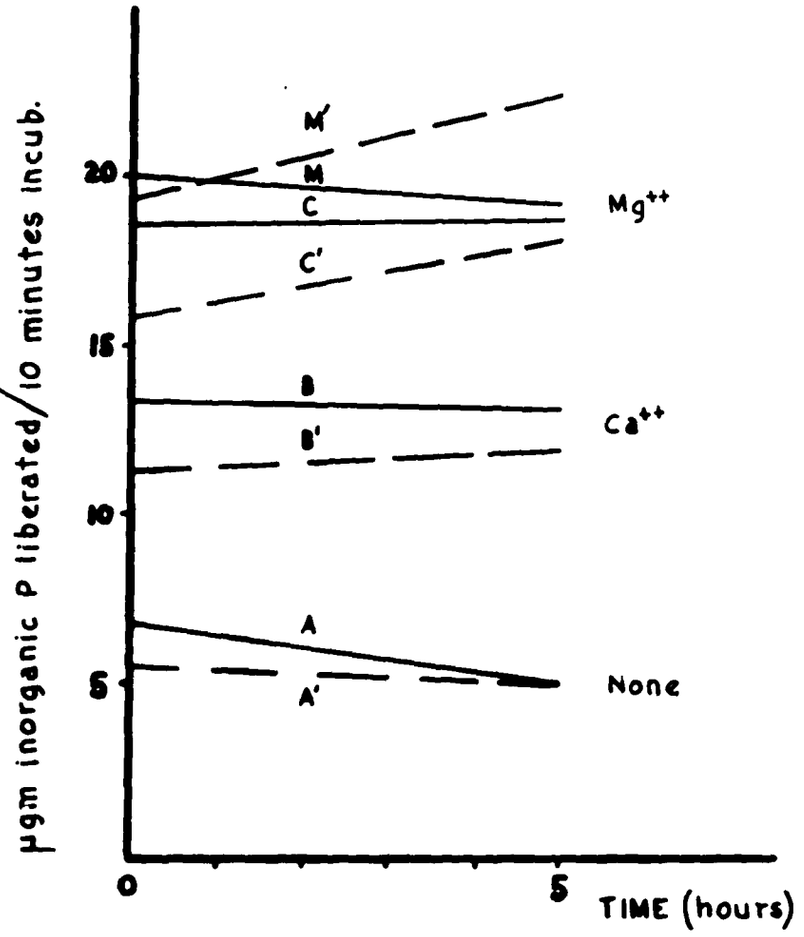
μgm inorganic P liberated/10 minutes incub.
20
15
10
5
0
5
TIME (hours)
M'
M
C
C'
B
B'
A
A'
Mg++
Ca++
None

TABLE 6

Effects of irradiation on oxygen consumption of Frog muscles

C = controls
I = irradiated

OXYGEN UPTAKE IN 10^{-6} MO_2/gm muscle.hr							
C		I		$C_{2.5K}$ / C_{0K}	$I_{2.5K}$ / I_{0K}	I / C	
0mMK	2.5mMK	0mMK	2.5mMK			0mMK	2.5mMK
2.45	3.87	4.78	8.04	1.56	1.68	1.95	2.10

FIGURE 6

Oxygen consumption of Frog muscles as a function of time and external concentration of K^+

--- 0mMK; o = controls; Δ = irradiated
—— 2.5mMK; . = controls; x = irradiated
I S.E.M.

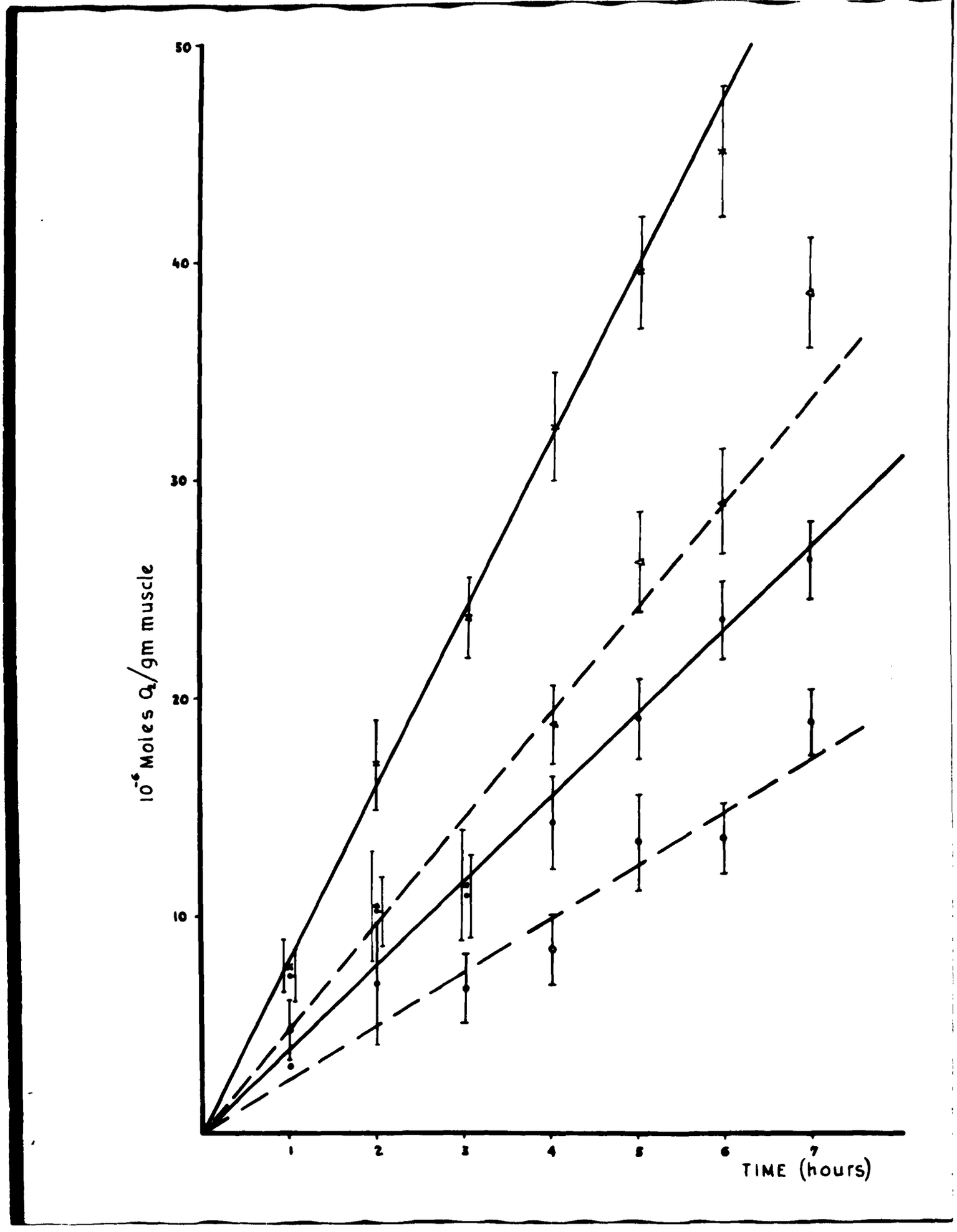
50
40
30
20
10
10^{-6} Moles O_2/gm muscle
1
2
3
4
5
6
7
TIME (hours)

TABLE 7

Oxidative phosphorylation in mitochondrial fractions prepared from control and irradiated frog muscles

P/O IN INORGANIC P UPTAKE (μATOMS)/OXYGEN UPTAKE (μATOMS)	
CONTROL	IRRADIATED
1.26	0.90
1.80	1.30
1.51	1.21
2.04	1.74

FIGURE 7

Proteolytic activity of Frog muscles as a function of time

C = controls
R = irradiated
$\underline{T}$ = S.E.M.

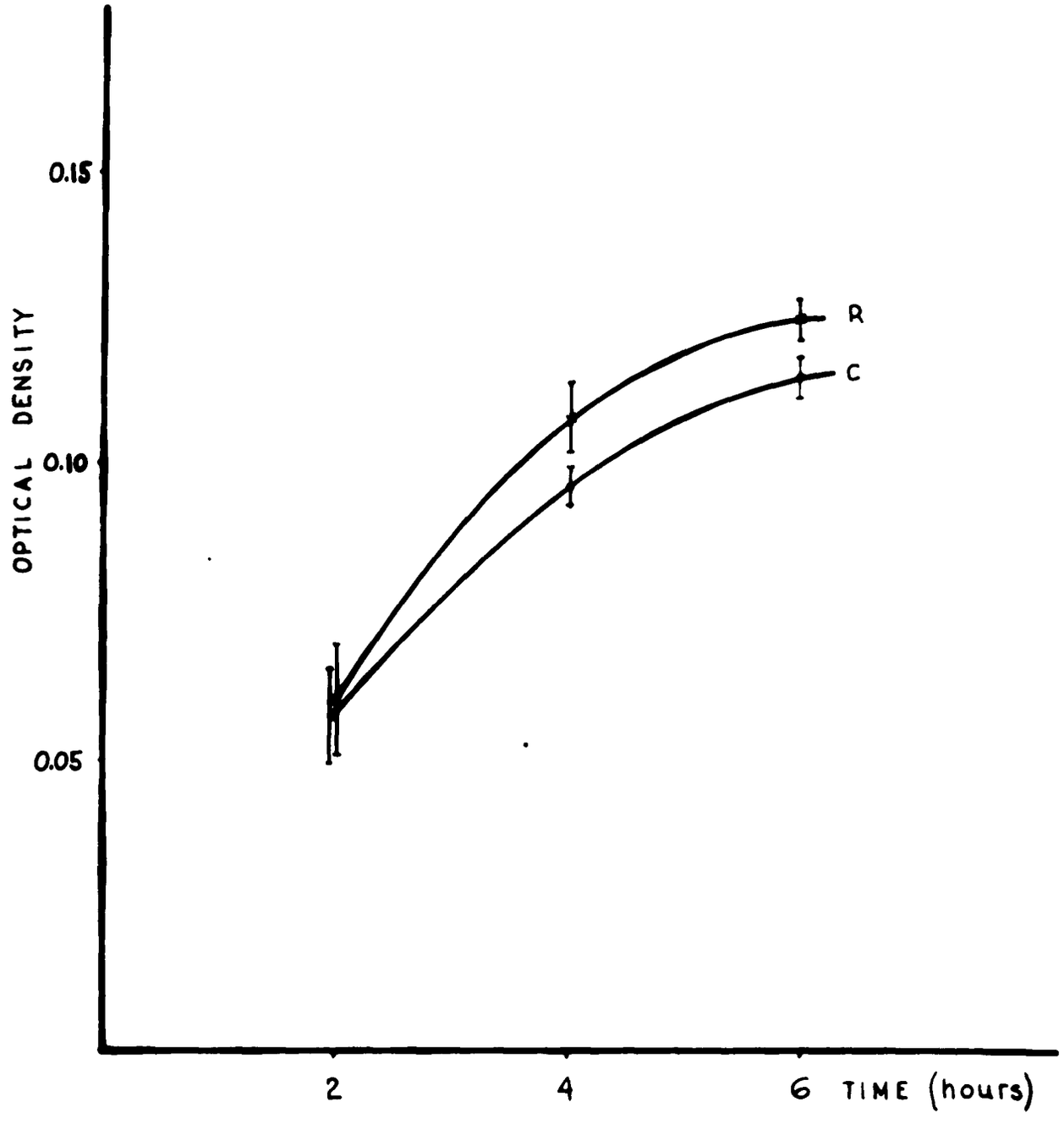
0.15
0.10
0.05
OPTICAL DENSITY
R
C
2
4
6
TIME (hours)

TABLE 8

Succinic dehydrogenase activity of Frog muscles

C = controls
I = irradiated

SUCCINIC DEHYDROGENASE ACTIVITY IN UNITS OF $\mu M O_2$ CONSUMED												
TIME (min)	10		20		30		40		50		60	
mg tissue/ml reaction med.	C	I	C	I	C	I	C	I	C	I	C	I
11	0.19	0.12	0.35	0.29	0.50	0.42	0.57	0.57	0.78	0.71	0.90	0.83
16	0.24	0.25	0.46	0.49	0.67	0.66	0.85	0.85	1.03	1.01	1.21	1.16
28	0.36	0.36	0.67	0.69	0.97	0.97	1.25	1.25	1.51	1.52	1.74	1.74

TABLE 9

ATP content of Frog muscles

C = controls
I = irradiated

mg of ATP per gm of muscle tissue															
LOAD IN GRAMS															
32				75				150				300			
C		I		C		I		C		I		C		I	
REST.	STI M	REST	STI M.	REST.	STIM.	REST.	STIM.	REST.	STIM.	REST.	STIM.	REST.	STIM.	REST.	STIM.
2.1	2.2	1.8	1.6	2.0	1.9	1.7	1.5	2.4	2.3	1.9	1.7	1.9	1.7	1.5	1.2

TABLE 10

Glycogen content in Frog muscles

C = controls
I = irradiated

GLYCOGEN CONTENT - mg/gm muscle			
NON-STIMULATED		STIMULATED	
C	I	C	I
8.6	7.5	7.7	6.0
9.1	7.9	8.6	6.4
8.5	6.4	7.5	5.1
6.2	6.0	6.3	4.3
7.8	6.8	6.6	4.7

TABLE 11

Oxygen consumption in the Frog's sartorius muscle in relation to Na^+ secretion.

C = controls
I = irradiated

CORRELATION BETWEEN Na^+ EFFLUX AND OXYGEN UPTAKE						
Na^+ efflux in 10^{-9} M.cm^{-2}.hr^{-1}		Oxygen uptake in 10^{-9} M.cm^{-2}.hr^{-1}		Ratio of Na^+ efflux to O_2 uptake		$\frac{\text{Irrad.}}{\text{Contr.}}$
C	I	C	I	C	I	
2.52	3.53	4.90	9.70	5.14	3.64	0.71